L8 ANSWER 17 OF 37 MEDLINE
AN 95403464 MEDLINE
DN 95403464
TI 73-kDa heat shock cognate protein interacts directly with the N-terminal region of the **retinoblastoma** gene product **pRb**. Identification of a novel region of **pRb**-mediating protein interaction.
AU Inoue A; Torigoe T; Sogahata K; Kamiguchi K; Takahashi S; Sawada Y; Saijo M; Taya Y; Ishii S; Sato N; et al
CS Department of Pathology, Sapporo Medical University School of Medicine, Japan..
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Sep 22) 270 (38) 22571-6.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199513
AB **Retinoblastoma** protein (**pRb**) functions as a tumor suppressor, and certain proteins are known to bind to **pRb** in the C-terminal region. Although the N-terminal region of **pRb** may also mediate interaction with some proteins, no such protein has been identified yet. We demonstrated previously the in vivo protein association between **pRb** and 73-kDa heat shock cognate protein (hsc73) in certain human tumor cell lines. In this report we analyzed the interaction between these two proteins in vitro. Our data showed that hsc73 interacts with the novel N-terminal region of **pRb**; that is, **pRb** binds directly to hsc73 and dissociates from hsc73 in an ATP-dependent manner. By using **deletion mutants** of cDNA encoding **pRb**, the hsc73 binding site of **pRb** was determined to be located in the region (residues 301-372) outside the so-called A pocket (residues 373-578) of this tumor suppressor protein. This finding was compatible with the fact that the adenovirus E1A oncoprotein, which is known to bind to the E2F binding pocket region of **pRb**, could not compete with hsc73 for the binding. Furthermore, phosphorylation of **pRb** by cyclin-dependent kinase inhibited the binding of **pRb** to hsc73. These data suggest that hsc73 may act exclusively as the molecular chaperone for nonphosphorylated **pRb**. As a result, hsc73 may function as a molecular stabilizer of nonphosphorylated **pRb**.

L [illegible] ANSWER 19 OF 37 MEDLINE
AN 94316219 MEDLINE
DN 94316219
TI Complex formation between lamin A and the **retinoblastoma** gene product: identification of the domain on lamin A required for its interaction.
AU Ozaki T; Saijo M; Murakami K; Enomoto H; Taya Y; Sakiyama S
CS Division of Biochemistry, Chiba Cancer Center Research Institute, Japan.
SO ONCOGENE, (1994 Sep) 9 (9) 2649-53.
Journal code: ONC. ISSN: 0950-9232.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199411
AB The **retinoblastoma** susceptibility gene product (**pRB**) has been known to function as a negative regulator of cell growth. Recent observations suggest that its biological activity might be modulated by an interaction with nuclear structures. By using in vitro binding assays, we have found that **pRB** can associate with lamin A, which has been known to be one of the major nuclear matrix proteins. A series of GST-lamin A **deletion mutants** was constructed to define

the amino acid sequence required for binding to **pRB**. A GST-lamin A (247-355) contained an activity to associate with **pRB**, while the other constructs such as GST-lamin A (37-244) or GST-lamin A (356-571), could not bind to **pRB**. Within the **pRB**-binding domain of lamin A, there exists the short amino acid sequence which is also present in the **pRB**-binding region of the transcription factor E2F-1. The similar experiments using a set of GST-RB **deletion mutants** revealed that a region containing the E1A binding pocket B and the carboxy-terminal portion of **pRB** was responsible for binding to lamin A.

L8 ANSWER 25 OF 37 MEDLINE
AN 94175085 MEDLINE
DN 94175085
TI Identification of a novel **retinoblastoma** gene product binding site on human papillomavirus type 16 E7 protein.
AU Patrick D R; Oliff A; Heimbrook D C
CS Department of Cancer Research, Merck Research Laboratories, West Point, Pennsylvania 19486.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Mar 4) 269 (9) 6842-50.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199406
AB Transformation of mammalian cells by human papillomavirus type 16 appears to require binding of the viral E7 protein to the cellular **retinoblastoma** growth suppressor gene product (**pRB**). Binding of E7 protein to **pRB** inhibits several of **pRB**'s biochemical properties, including association with the transcription factor E2F. Fragments of E7 protein derived from its conserved region 2 (CR2) domain bind to **pRB** and are sufficient to inhibit binding of full-length E7 protein to **pRB**. However, these CR2 fragments exhibit reduced affinity for **pRB** compared to the full-length protein and do not inhibit formation of the **pRB**-E2F complex. These observations suggest the existence of additional contact sites between the E7 protein and **pRB**. In the current study we have identified a region of E7, distinct from the CR2 domain, which is sufficient to bind **pRB**. This new **pRB** binding motif encompasses the zinc-binding conserved region 3 (CR3) domain of E7. Studies with a series of **pRB deletion mutants** suggest that **pRB** residues between amino acids 803 and 841 are necessary for binding to the E7 CR3 domain. An E7 CR3 peptide inhibits binding of E2F to **pRB**, indicating that E2F and E7(31-98) bind to **pRB** at the same or overlapping sites. These results are consistent with a model in which optimal binding of E7 to **pRB** requires at least two distinct contact sites: the previously identified high affinity interaction between the E7 CR2 domain and the **pRB** "pocket" region, and a second interaction between the E7 CR3 domain and the COOH-terminal region of **pRB**. The latter interaction is sufficient for E7's inhibition of E2F binding to **pRB**.

L8 ANSWER 26 OF 37 MEDLINE
AN 93103736 MEDLINE
DN 93103736
TI Biological function of the **retinoblastoma** protein requires distinct domains for hyperphosphorylation and transcription factor binding.
AU Qian Y; Luckey C; Horton L; Esser M; Templeton D J
CS Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106.
NC CA 38719 (NCI)
SO MOLECULAR AND CELLULAR BIOLOGY, (1992 Dec) 12 (12) 5363-72.
Journal code: MCY. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199303
AB Despite the importance of the **retinoblastoma** susceptibility gene to tumor growth control, the structural features of its encoded protein

pRb) and their relationship to protein function have not been well explored. We constructed a panel of **deletion mutants** of **pRb** expression vectors and used a biological assay for **pRb** that measures growth inhibition and morphologic changes in **pRb**-transfected Saos-2 cells to correlate structural alterations of the **pRb** coding region with function. We tested the deleted proteins for the ability to bind to viral oncoprotein E1A and to the transcription factor E2F. We also measured the ability of the mutant proteins to become hyperphosphorylated in vivo and to be recognized as substrates in vitro by a cell cycle-regulatory kinase associated with cyclin A. We identified two regions of **pRb** that are required for E2F binding and for hyperphosphorylation. E1A binding domains partially overlap but are distinct from both of these other two regions. Biological function of **pRb** is dependent on retention of the integrity of both of these biochemically defined domains. These data support the model that **pRb** is a transducer of afferent signals (via the kinase that phosphorylates it) and efferent signals (through transcription factor binding), using distinct structural elements. Preservation of both of these features is essential for the ability of **pRb** to induce growth inhibition and morphologic changes upon reintroduction into transfected cells.

LS ANSWER 31 OF 37 MEDLINE
AN [illegible] MEDLINE
DN [illegible]
TI Hyperphosphorylation of the **retinoblastoma** gene product is determined by domains outside the simian virus 40 large-T-antigen-binding regions.
AU Hamel P A; Cohen B L; Sorce L M; Gallie B L; Phillips R A
CS Division of Immunology and Cancer, Hospital for Sick Children Research Institute, Toronto, Ontario, Canada..
SO MOLECULAR AND CELLULAR BIOLOGY, (1990 Dec) 10 (12) 6586-95.
Journal code: MCY. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM [illegible]
AB With the murine **retinoblastoma** (RB) cDNA, a series of RB mutants were expressed in COS-1 cells and the **pRB** products were assessed for their ability (i) to bind to large T antigen (large T), (ii) to become modified by phosphorylation, and (iii) to localize in the nucleus. All point mutations and deletions introduced into regions previously defined as contributing to binding to large T abolished **pRB**-large T complex formation and prevented hyperphosphorylation of the RB protein. In contrast, a series of deletions 5' to these sites did not interfere with binding to large T. While some of the 5' **deletion mutants** were clearly phosphorylated in a cell cycle-dependent manner one, delta Pvu, failed to be phosphorylated depsite binding to large T. **pRB** with mutations created at three putative p34cdc2 phosphorylation sites in the N-terminal region behaved similarly to wild-type **pRB**, whereas the construct delta P5-6-7-8, mutated at four serine residues C terminal to the large T-binding site, failed to become hyperphosphorylated despite retaining the ability to bind large T. All of the mutants described were also found to localize in the nucleus. These results demonstrate that the domains in **pRB** responsible for binding to large T are distinct from those recognized by the relevant **pRB**-specific kinase(s) and/or those which contain cell cycle-dependent phosphorylation sites. Furthermore, these data are consistent with a model in which cell cycle-dependent phosphorylation of **pRB** requires complex formation with other cellular proteins.